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next generation protein therapeutics

Single domain antibodies against CXCR4 as a potential therapy in retinal vascular disease

Discovery on Target, Boston 2016: *Treating Ocular Disorders*

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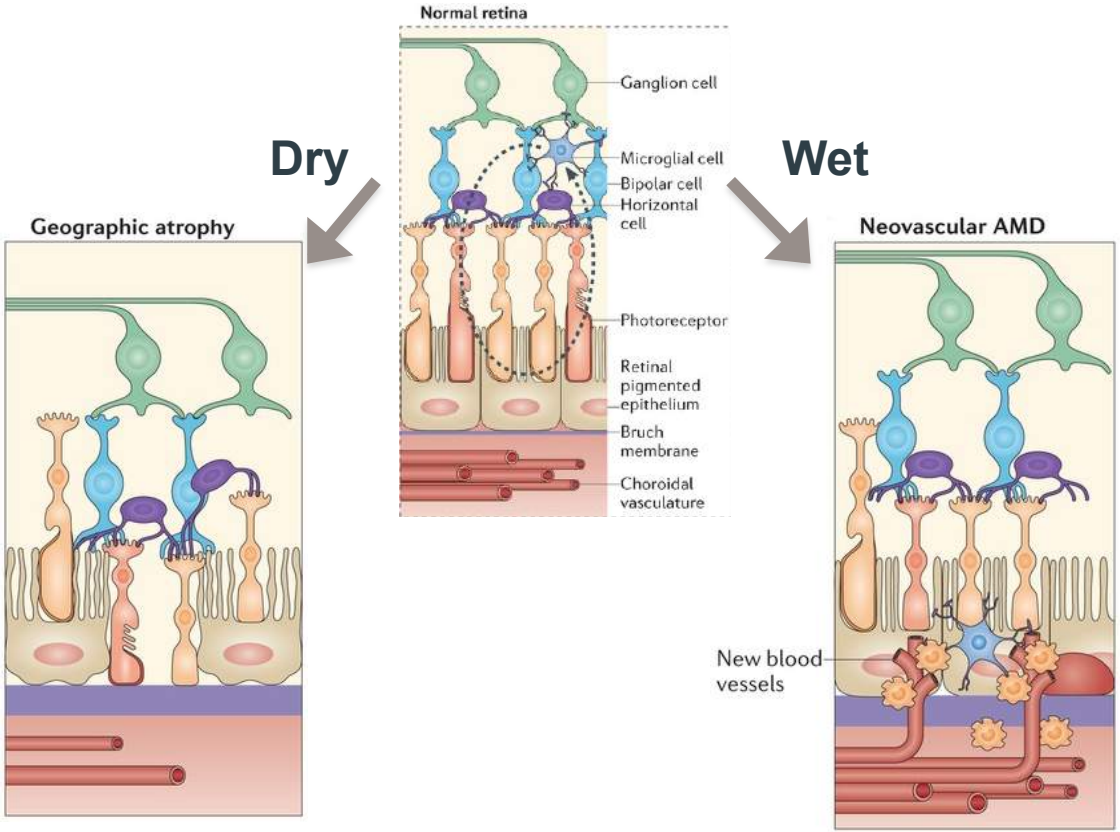
Age-related macular degeneration (AMD)



Geographic atrophy (Dry AMD)

- contraction of choroidal blood vessels
- photoreceptors breakdown

Neovascular AMD (Wet AMD)

- abnormal choroidal blood vessel growth
- blood vessel invade under the retina and macular
- blood vessel bleeding and fluid leakage

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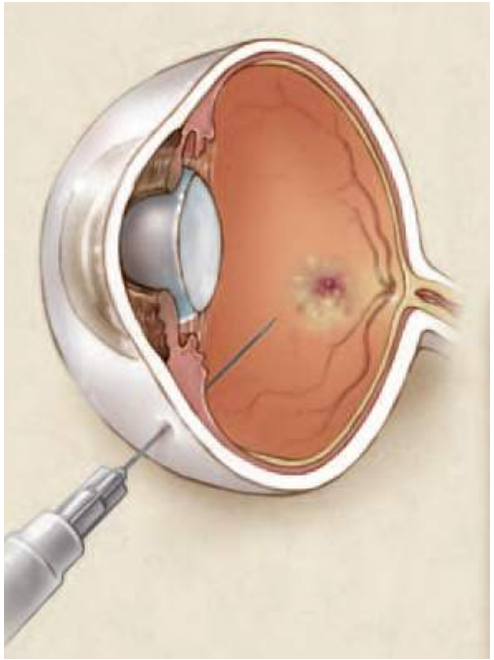
Treatment for AMD

Current treatments for AMD target the role of VEGF in abnormal blood vessel growth:

- Ranibizumab (Lucentis®)
- Bevacizumab (Avastin®)
- Aflibercept (Eylea®)



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 5, 2006 VOL. 355 NO. 14

Ranibizumab for Neovascular Age-Related Macular Degeneration

Philip J. Rosenfeld, M.D., Ph.D., David M. Brown, M.D., Jeffrey S. Heier, M.D., David S. Boyer, M.D., Peter K. Kaiser, M.D., Carol Y. Chung, Ph.D., and Robert Y. Kim, M.D., for the MARINA Study Group*

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 19, 2011 VOL. 364 NO. 20

Ranibizumab and Bevacizumab for Neovascular Age-Related Macular Degeneration

The CATT Research Group*

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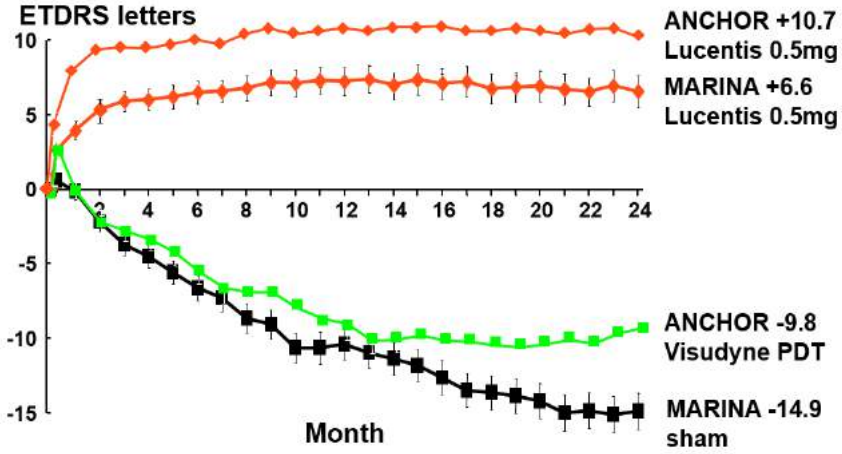
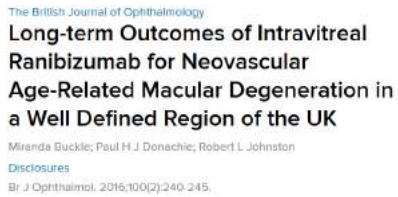
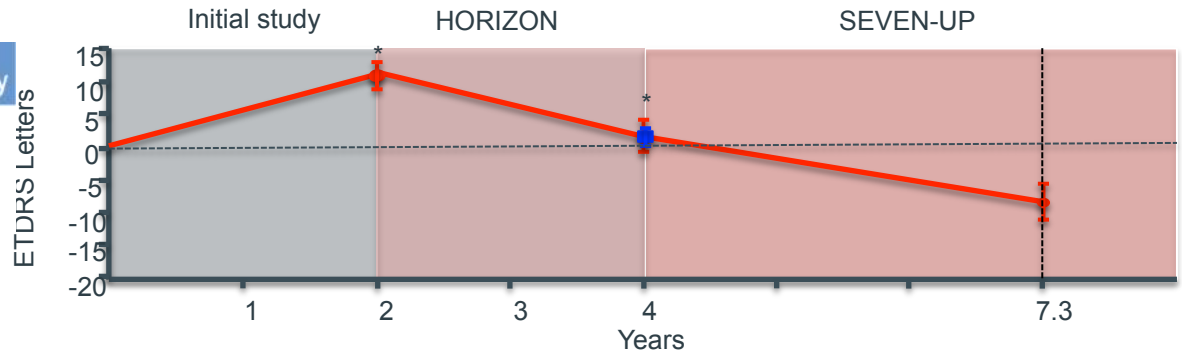
Limitation of current treatment



Despite an improvement with current therapies there is still long term deterioration of vision

There is a need for new treatments that avoid intravitreal delivery (eye drops) and that target alternatives to VEGF





Rosenfeld et al, N Engl J Med 2006; 355(14): 1419. Brown et al, N Engl J Med 2006; 355(14); 1432

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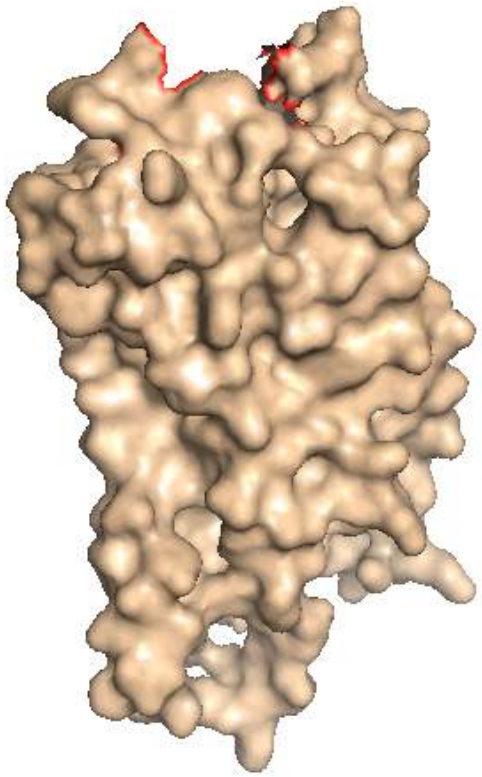
CXCR4 is involved in fibrosis and other disease states

CXCR4 is important in maintaining stem cells in bone marrow with Mozobil (AMD3100) approved for single use.

HIV-1 uses CXCR4 as a co-receptor for viral entry into host cells and CXCR4 has been associated with more than 23 types of cancers

CXCR4 has more recently been recognised as a critical player in development of a number of areas of fibrosis including:



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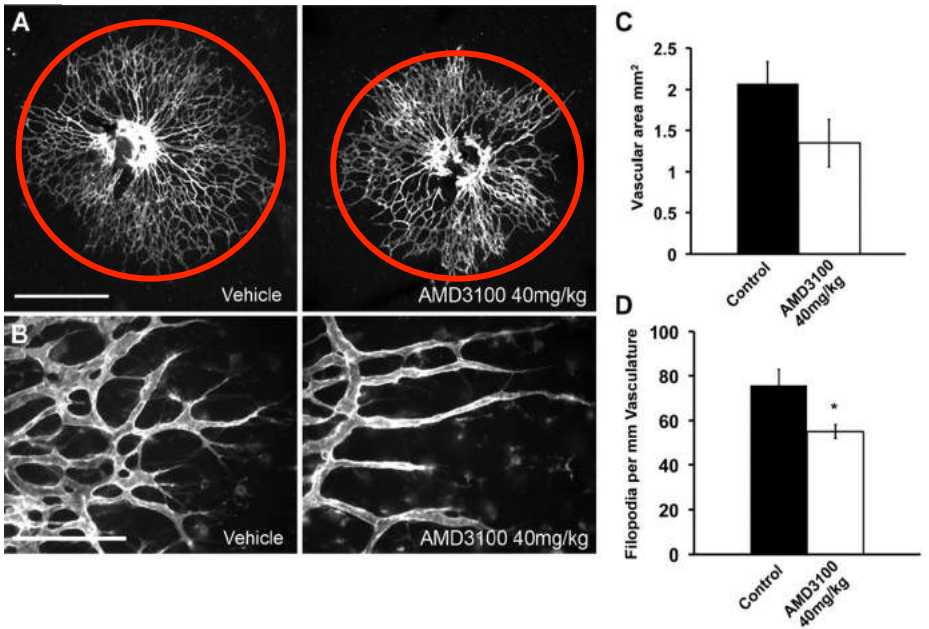
Role of CXCR4 in retinal disease

Several reports have suggested that the CXCR4 antagonist AMD3100 was able to suppress retinal neovascularization

AdAlta has identified i- body antagonists of CXCR4

The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization

Raquel Lima e Silva,* Jikui Shen,* Sean F. Hackett,* Shu Kachi,* Hideo Akiyama,* Katsuji Kiuchi,* Katsutoshi Yokoi,* Maria C. Hatara,* Thomas Lauer,* Sadia Aslam,* Yuan Yuan Gong,* Wei-Hong Xiao,* Naw Htee Khu,* Catherine Thut,† and Peter A. Campochiaro*,‡



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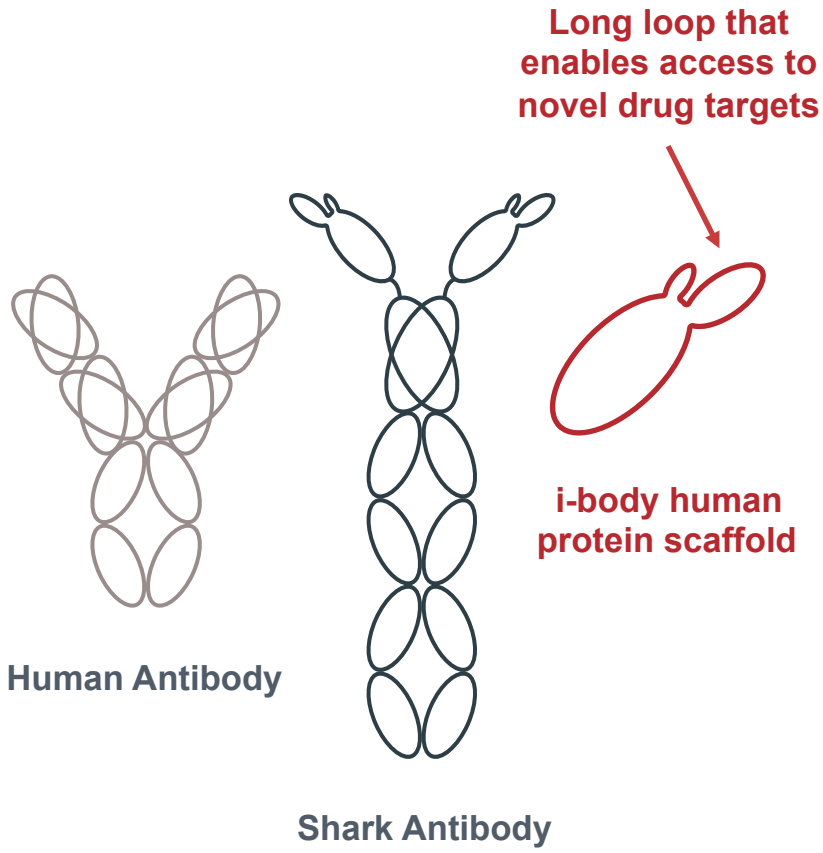
i-body technology

AdAlta is developing a new technology platform that produces unique proteins known as i-bodies, that mimic the shape of shark antibody binding domain and engineers their key stability features into a human protein, for therapeutic intervention in disease.

The single domain antigen binding region of shark antibodies is extremely stable and has a long binding loop not present in either human or next generation antibodies.

Advantages of i-bodies

- High target specificity and high affinity for their target
- Small proteins; 10% the size of a typical human antibody
- Highly stable to proteases, high temperatures and low pH
- Long loop that can bind to a diverse range of therapeutically relevant targets including those that are difficult for current antibody therapies
- **Human protein** – reduced risk of immune response



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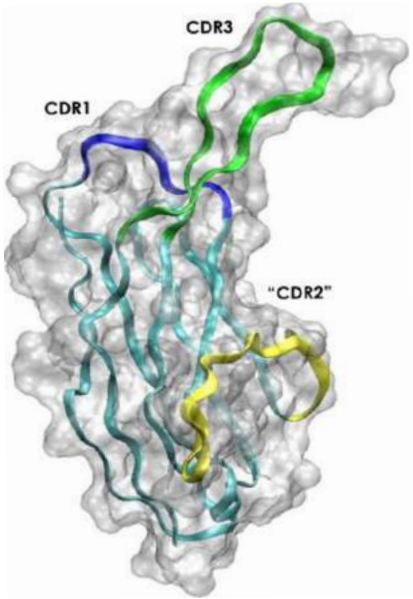
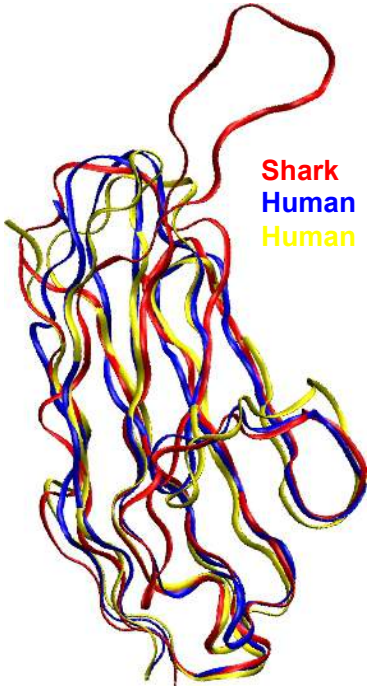
i-bodies: human single domains

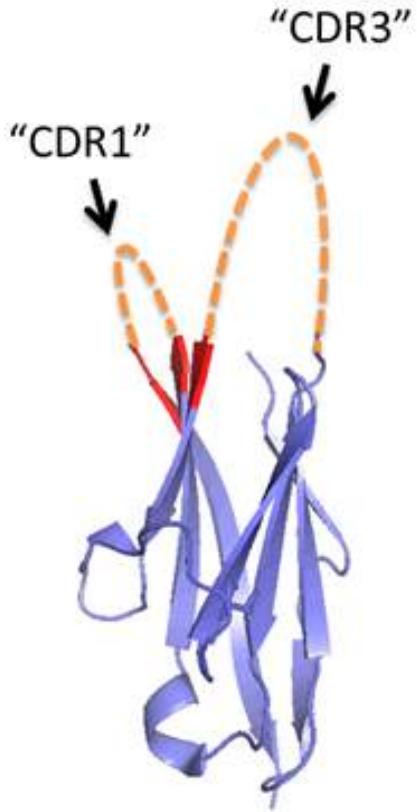


VNAR



Ribbon Overlay

NCAM Domain 1



i-body library

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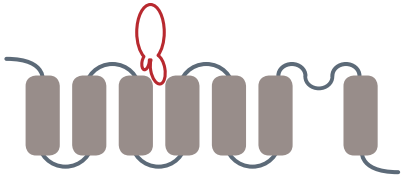
i-body technology advantages

Challenging targets

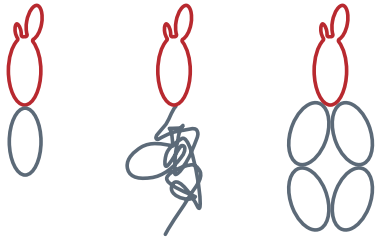


Because of the long binding loop of the i-body, that is lacking in traditional antibodies, i-bodies recognise and bind to a diverse range of different therapeutically-relevant targets including those that are difficult/intractable to access by current antibody therapies such as G-protein coupled receptors (GPCRs) and ion channels.

Multiple delivery routes

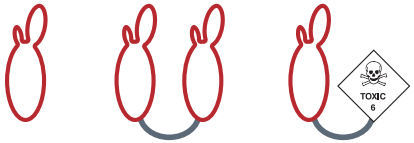
The small physical size and stable properties of i-bodies provides advantages for tissue and organ penetration as well as multiple delivery routes.

Customised half-life



As a result of their small size and exceptional stability i-bodies can serve as building blocks to engineer therapeutics with tailored pharmacokinetic properties.

Multi formatting



Can easily engineer unique differentiated i-body products in a variety of formats including monospecific and bispecifics as well as i-body drug conjugates (IDCs), thus tailoring them for different therapeutic purposes.

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i-bodies combine benefits of small molecules and conventional antibodies

	Small Molecule	Conventional Antibody	AdAlta i-body
High selectivity-specificity		●	●
Low toxicity: no off target effects		●	●
Cavity binding and new epitopes	●		●
Stability	●		●
Alternative routes of administration	●		●
Easy to manufacture	●		●
Speed & risk of development		●	●

Long loop that enables access to novel drug targets

i-body human protein scaffold

i-bodies offer a new and potentially more effective approach to the treatment of a wide range of human diseases.

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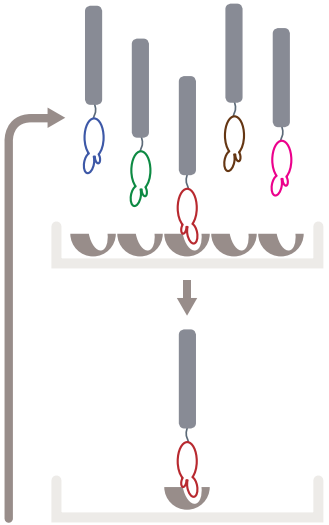
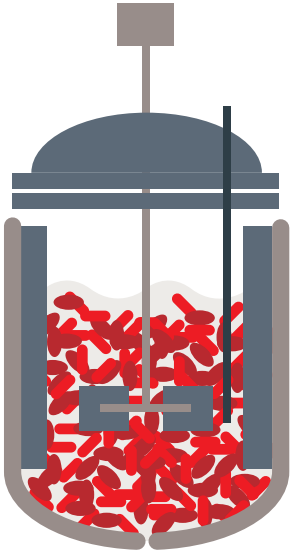
Selecting i-bodies against CXCR4

Large diverse synthetic library of 2 billion i-body protein compounds that can bind to a broad range of therapeutically relevant targets



i-body identified by rapid screening on CXCR4 lipoparticles

i-body affinity matured to enhance target binding and generate lead i-body candidate



Manufactured in microbial systems; more cost-effective and easier than conventional monoclonal antibodies. Potential for direct peptide synthesis.

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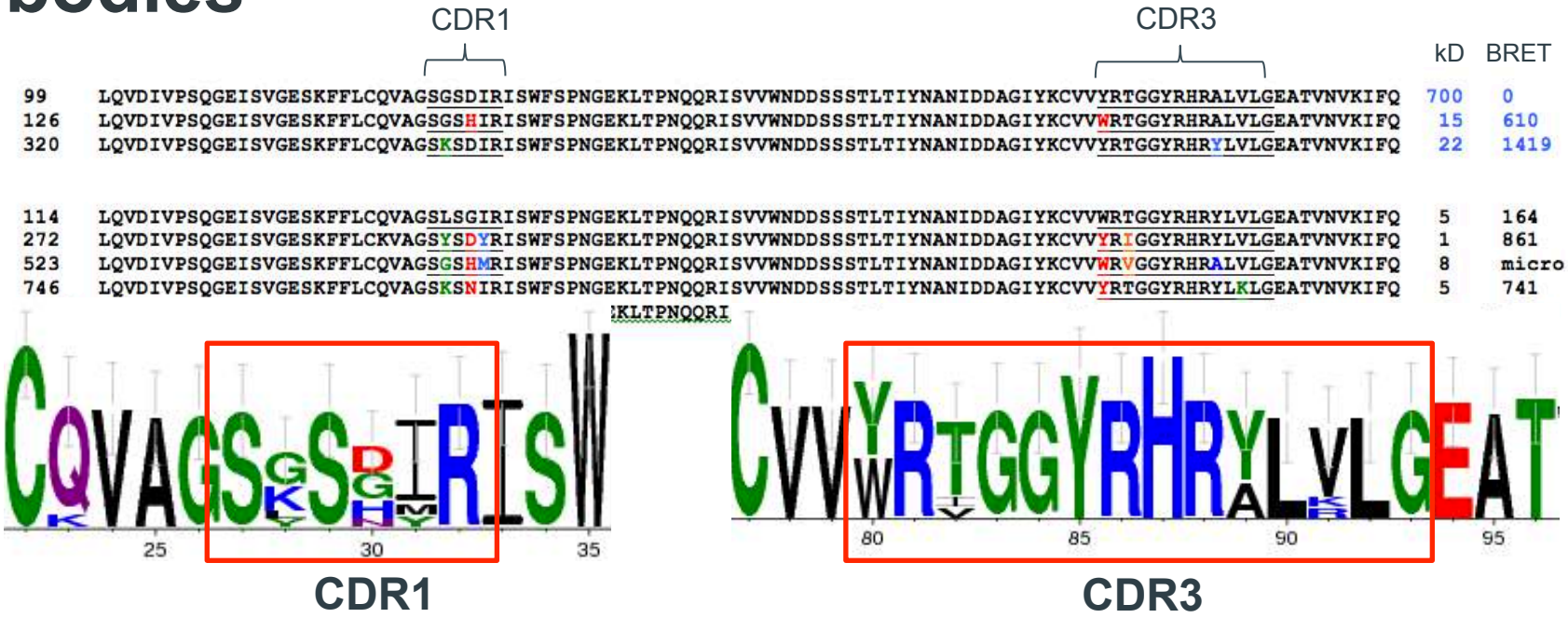
Sequences of affinity matured CXCR4 binding i-bodies



The affinity maturation process allowed identification of residues in the CDR1 and CDR3 that were important for binding to CXCR4.

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Family of CXCR4 binding i-bodies

i-bodies from the affinity maturation process were evaluated in several *in vitro* assays including

- β-arrestin BRET assay
- Calcium flux assay
- cAMP assay

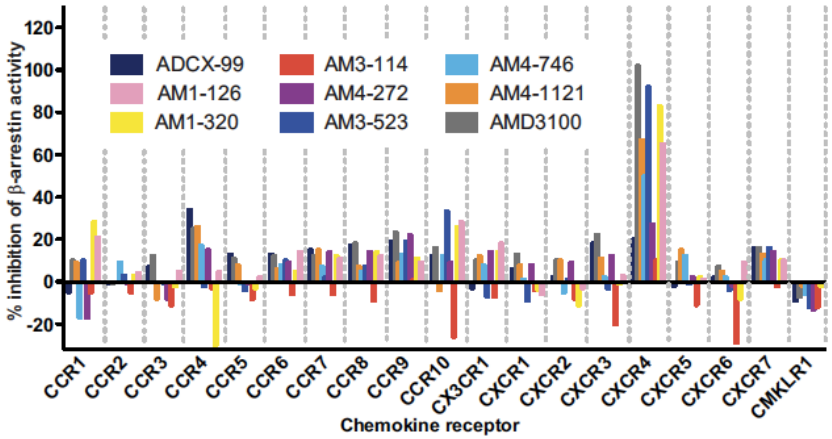
1-3 single point mutations could dramatically effect the β-arrestin activity, while still having the same affinity for the receptor CXCR4

None of the i-bodies identified had any effect on calcium flux

Protein	Affinity to CXCR4 (nM)	IC50 (nM) in b-Arrestin BRET assay
ADCX-99	700	No activity
AD-320	21.8	1419
AD-245	14.88	~uM
AD-126	14.75	610
AD-466	13.83	6322
AD-661	9.53	1544
AD-523	8.55	12713
AD-613	7.83	1826
AD-1121	7.21	796
AD-920	7.18	~uM
AD-746	5	741
AD-114	4.85	164
AD-272	1.6	861

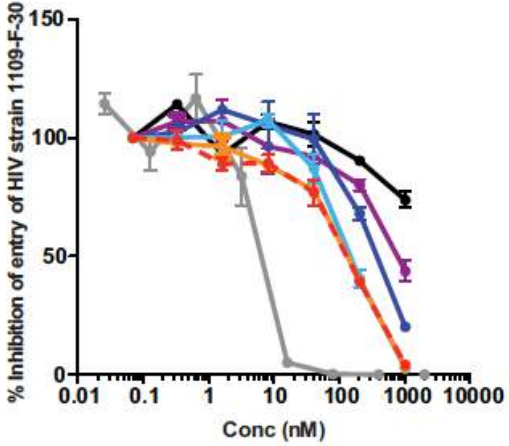
Panel of i-bodies against CXCR4 specific and have *in vitro* activity

I-bodies are specific to CXCR4



When tested against 167 GPCRs, the 10 affinity matured i-bodies specifically antagonized CXCR4. There was no agonist activity on any of the GPCRs.

I-bodies have *in vitro* activity



The i-bodies were evaluated in a number of *in vitro* assays. In this assay we demonstrated that a panel of i-bodies with affinity to CXCR4 <20nM could inhibit inhibition of entry of the HIV virus.

REF: Griffiths et al JBC June 2016

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Epitope mapping: core residues on CXCR4

CDR1 CDR3

```
114   LQVDIVPSQGEISVGESKFFLCQVAG SLSGIR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV WRTGGYRHRYLVLG EATVNVKIFQ
272   LQVDIVPSQGEISVGESKFFLCQVAG SYSDYR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV YRIGGYRHRYLVLG EATVNVKIFQ
523   LQVDIVPSQGEISVGESKFFLCQVAG SGSHMR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV WRVGGYRHRALVLG EATVNVKIFQ
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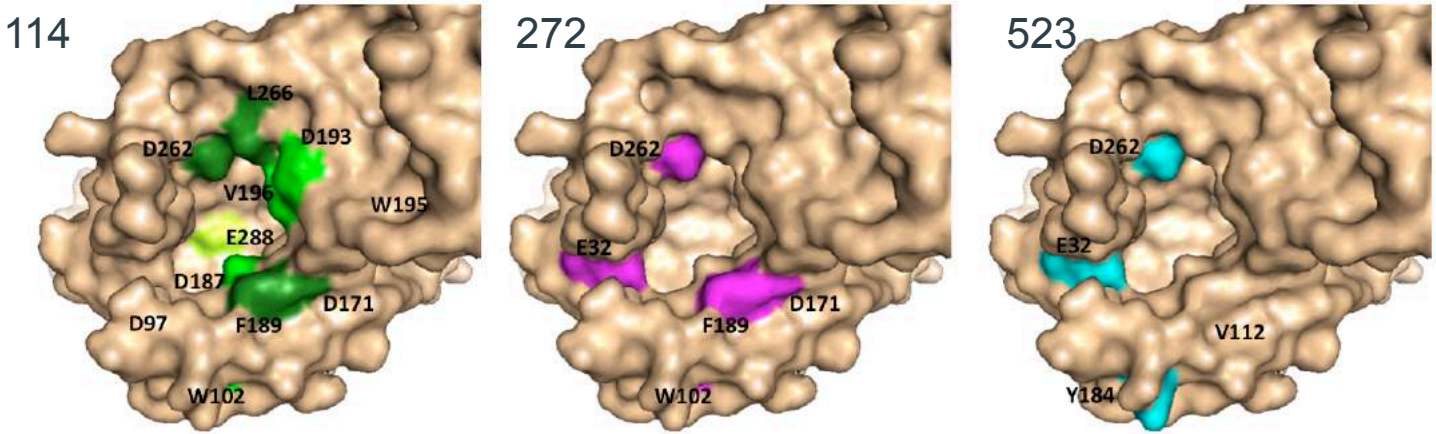

High β-arrestin
High cAMP

Low β-arrestin
High cAMP

Medium β-arrestin
Medium cAMP

Epitope mapping of three i-bodies to CXCR4, demonstrated that residue E262 was common to all i-bodies. E262 which is deep in groove of CXCR4, also binds AMD3100. All three i-bodies had unique binding sites reflecting their different functionalities.

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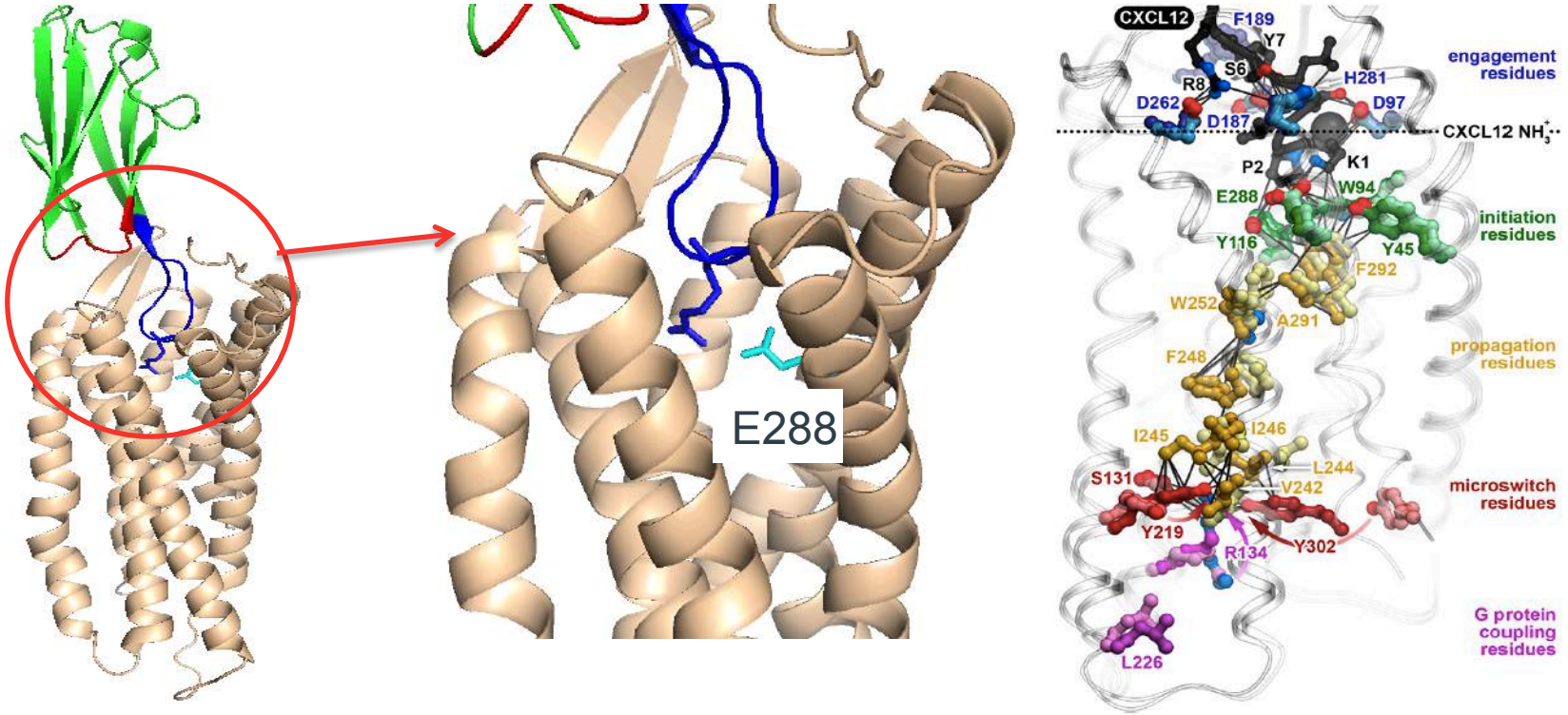
Homology model of i-body and CXCR4



Side chain CDR3 contact residues also demonstrate that the long loop of the i-body, binds deep in the ligand binding pocket of CXCR4

REF: Wescott et al PNAS 2016

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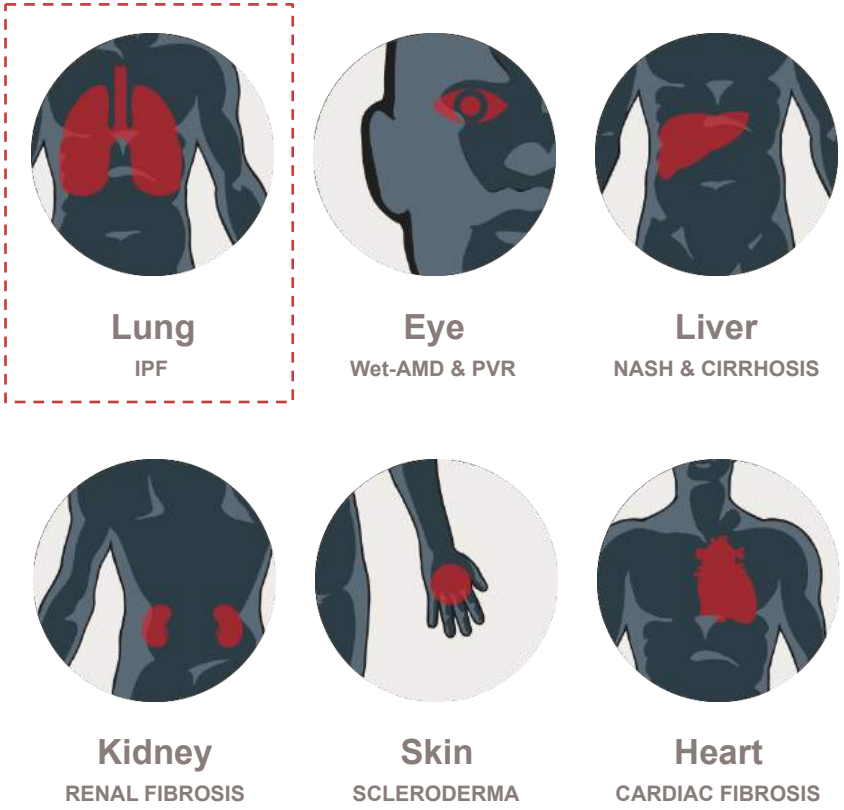
Fibrosis: AD114 i-body has anti-fibrotic activity in lung

- Developing i-bodies as improved therapies for the treatment of fibrosis
  - a condition that is prevalent in 45-50% of all diseases
- Fibrosis can occur in many tissues of the body as a result of inflammation or damage
  - it can result in scarring of vital organs causing irreparable damage and eventual organ failure
- AdAlta's initial focus is on lung fibrosis

Collectively fibrosis represents a large unmet clinical need



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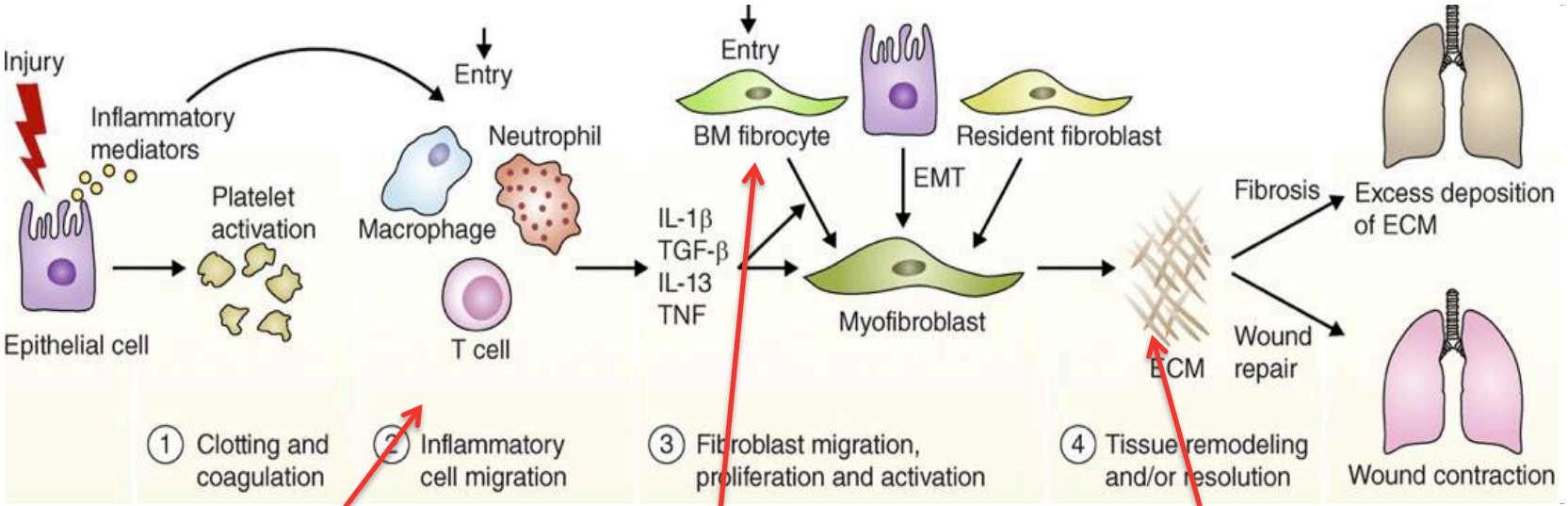
Fibrosis is a complex disease



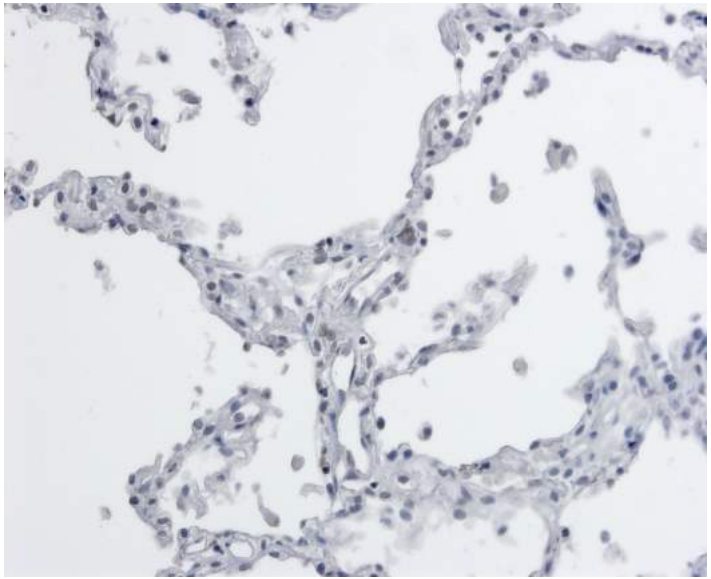
Inflammation

Fibrocyte migration

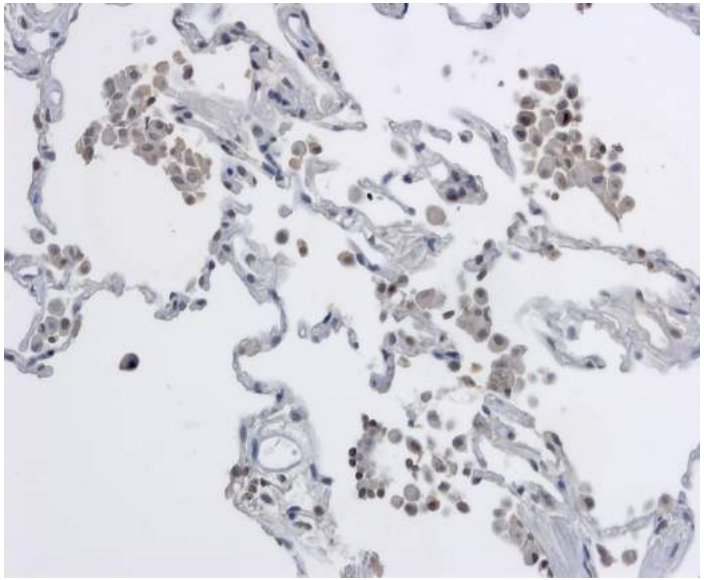
Deposition of collagen/ECM

AD-114 binds to lung tissue from patients with fibrosis

The i-body AD-114 was used for Immunohistochemical (IHC) staining of normal and diseased lung tissues to verify expression of CXCR4 *in situ*



AD-114 does not bind lung tissue from normal lungs



AD-114 binds to lung tissue from fibrosis

CEDARS-SINAI

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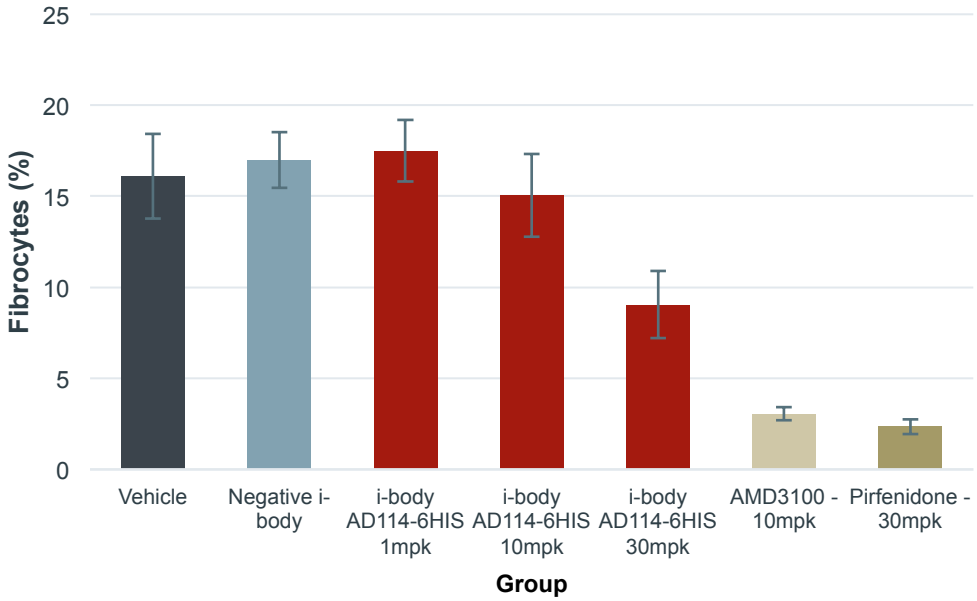
Bleomycin mouse model

Administration of Bleomycin is the most common animal model for the assessment of candidate drugs for the treatment of IPF. The Bleomycin treated mouse lung shows extensive collagen deposition and inflammatory cell infiltration.

- Mice received intratracheal instillation of Bleomycin at 2U/kg/mouse
- Groups treated starting on Day 0 of the study 1 hr prior to Bleomycin installation, with selected test compounds
- i-body was dosed at three levels,1mg/kg, 10mg/kg and 30mg/kg daily
- At Day 4 the number of fibrocytes (CXCR4+, Col1+ and CD45+ cells) in the lungs were measured by flow cytometry, RNA levels of collagen and collagen content were measured
- At Day 19 whole lungs were assayed for Hydroxyproline and histology (Masson's Trichrome) and Ashcroft score completed to analysis collagen content (Ashcroft, T., J.M. Simpson, and V. Timbrell. 1988. J. Clin. Pathol. 41:467-470). Body weights were also evaluated

AD-114 reduces fibrocytes in the Bleomycin mouse model

Mice challenged with Bleomycin and treated with AD-114 had reduced levels of fibrocytes in their lungs when compared to the mice treated with the negative control i-body



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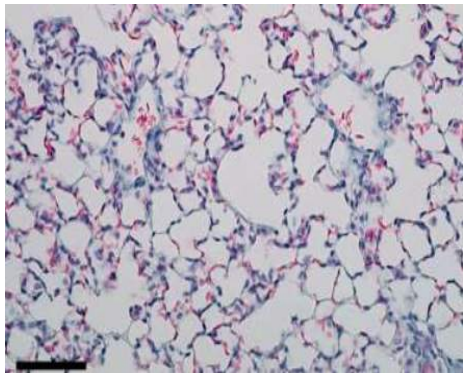
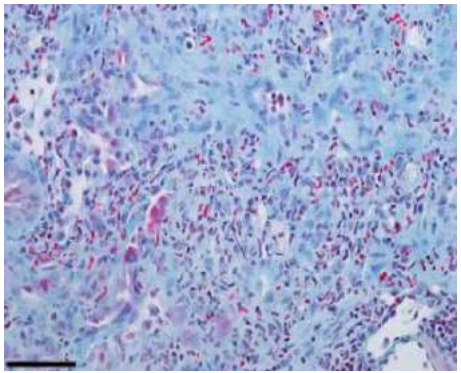
AD-114 prevents lung fibrosis in in the Bleomycin mouse model

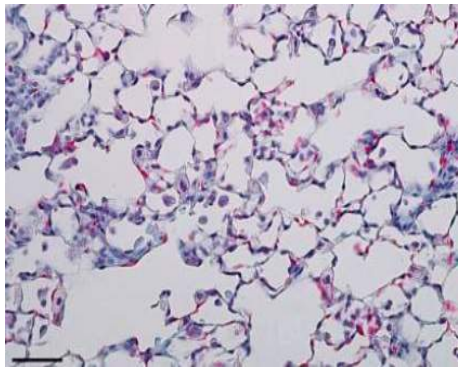
Extensive pre-clinical AD-114 studies have demonstrated positive *in vitro* (in the lab) and *in vivo* (in animals) data



Normal lung tissue



IPF lung tissue
(lung disease mouse model)



IPF lung tissue + AD-114 dosed for 21 days
(lung disease mouse model)

AD-114 reduces collagen content and inflammatory cell infiltration and demonstrates a similar architecture to that of the normal lung in the Bleomycin mouse model

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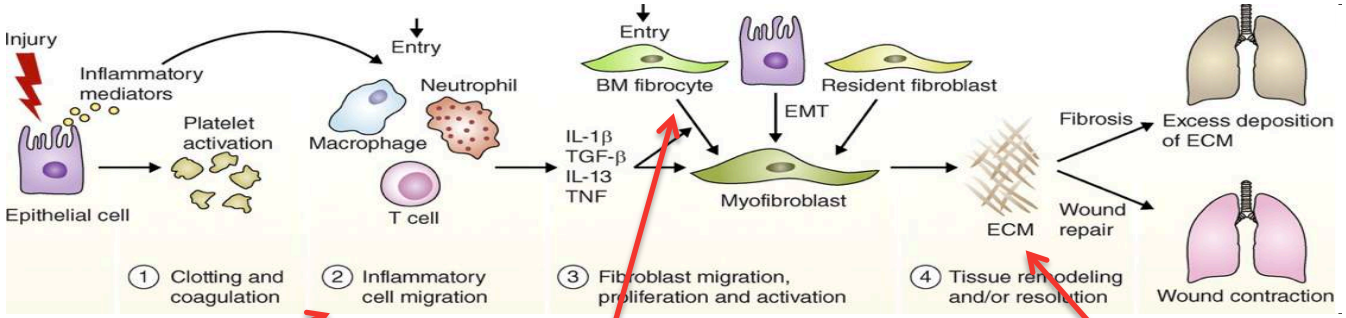
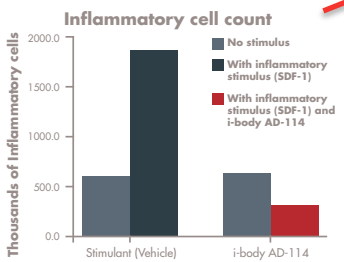
AD-114 reduces fibrocytes in the Bleomycin mouse model

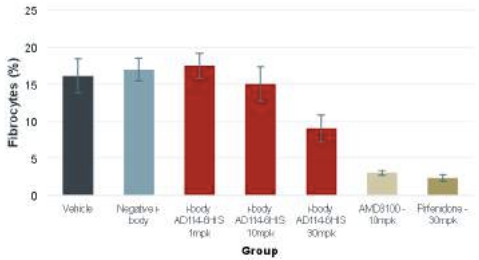
- AD-114 blocks SDF-1 induced infiltration of leukocytes in a mouse air pouch assay. Anti-inflammatory activity is important for preventing fibrosis.
- AD-114 specifically inhibited migration of slow and rapid IPF fibroblast migration but did not have any effect on normal fibroblasts with greater *in vitro* efficacy compared to the only approved therapies Nintedanib and Pirfenidone for IPF treatment
- Treatment with i-body AD-114 in the Bleomycin mouse model reduced collagen at the gene and the protein level
- The negative control i-body had no effect on collagen deposition
- Loss of body weight due to Bleomycin was prevented with daily dosing of i-body AD-114 10mg/kg
- i-bodies specifically inhibited migration of slow and rapid IPF fibroblast migration but did not have any effect on normal fibroblasts

AD-114 inhibits key features of the fibrogenic pathway with novel MOA

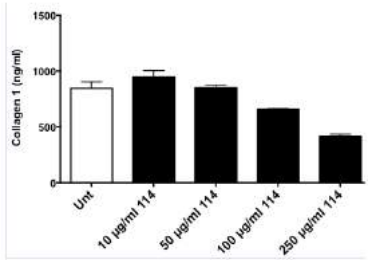




Modulate aspects of inflammation



Block fibrocyte recruitment into the damaged lung



Reduce ECM deposition during tissue remodeling

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Model 1: Laser induced CNV and fibrosis

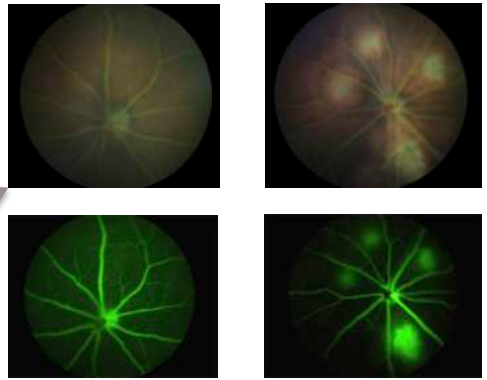
Laser treatment
(350mW, 532 CW laser)
& intravitreal injection
i-Body

Fluorescein angiography
Histology and mRNA

1 week

Laser: 532nm continuous wave laser

Untreated | Lasered



Analysis

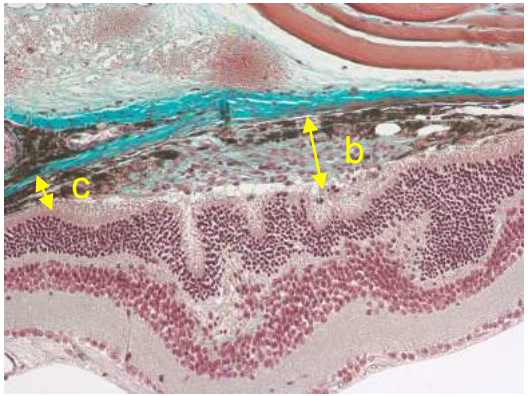
Lesions: % lesions that leak at 7 days
Size of FA lesion

Histology: Height of lesion relative to choroid.
Trichrome stain

IMCC: Iba1, Col1 and GFAP

Gene: Angiogenesis and fibrosis PCR array

Lesion height was quantified by measuring the height of the lesion (b), and normalising to the choroid (c) or b/c ratio.



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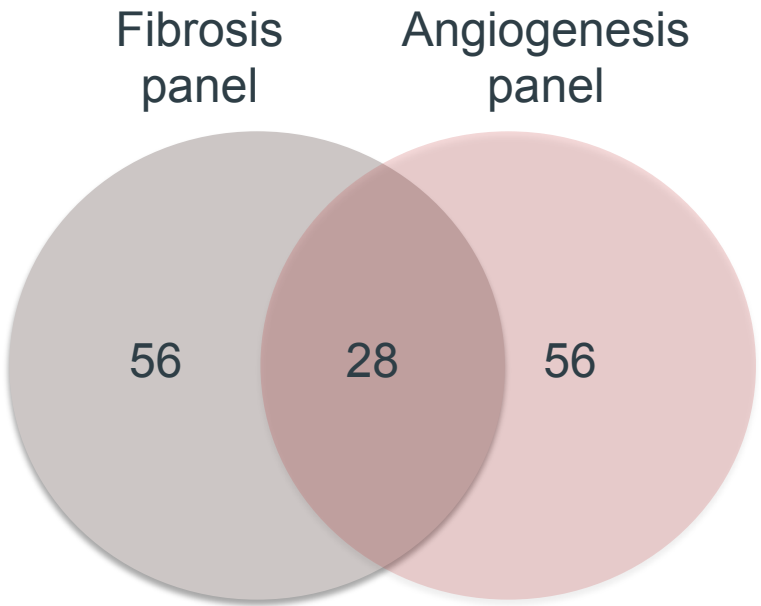
PCR array methodology

- Laser treated+/-i-bodies. mRNA collected 7 days later.
- mRNA extracted from posterior eye cup (ie RPE/Choroid)
- Fibrosis array (SABiosciences-Qiagen): 84 genes
- Angiogenesis array: 84 genes



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What genes are altered in laser induced CNV?

Inflammation: Ccl3, Itgb3

ECM remodeling: Mmp3, Mmp8, Timp1, Lox, Thbs1, Col1a2, Plat

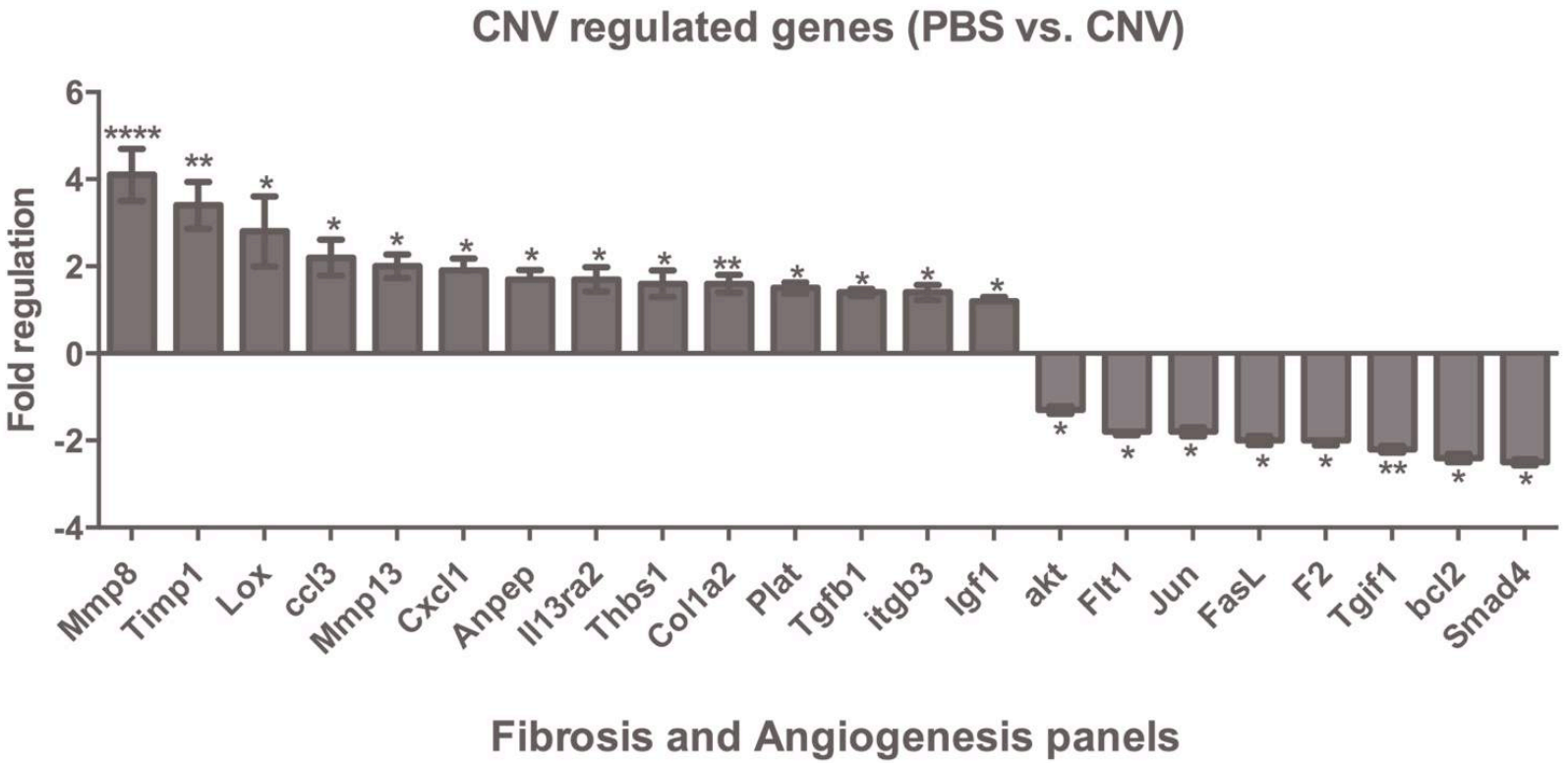
TGF−β: Tgfb1, FasL, Tgif1, smad4

Angiogenesis: Anpep, iL13ra2, Plat, Igf-1, Alt, Flt1, Jun, F2

Other fibrosis genes: bcl2

Rank	Gene ontological (GO) pathway	Total genes	Regulated Genes	Enrichment score	P-value
1	collagen catabolic process (GO:0030574)	22	4	> 100	1.09E-04
2	collagen metabolic process (GO:0032963)	37	4	92.81	8.65E-04
3	positive regulation of fibroblast proliferation (GO:0048146)	64	6	80.48	9.61E-07
4	regulation of fibroblast proliferation (GO: 0048145)	96	6	53.65	1.07E-05
5	SMAD protein signal transduction (GO: 0060395)	64	4	53.65	7.58E-03
6	transforming growth factor beta receptor signaling pathway (GO:0007179)	73	4	47.04	1.27E-02
7	response to hypoxia (GO:0001666)	175	6	29.43	3.69E-04
8	positive regulation of hemopoiesis (GO: 1903708)	177	6	29.1	3.94E-04
9	gliogenesis (GO:0042063)	189	5	22.71	1.95E-02
10	response to wounding (GO:0009611)	304	8	22.59	1.17E-05

Dysregulated genes in the RPE



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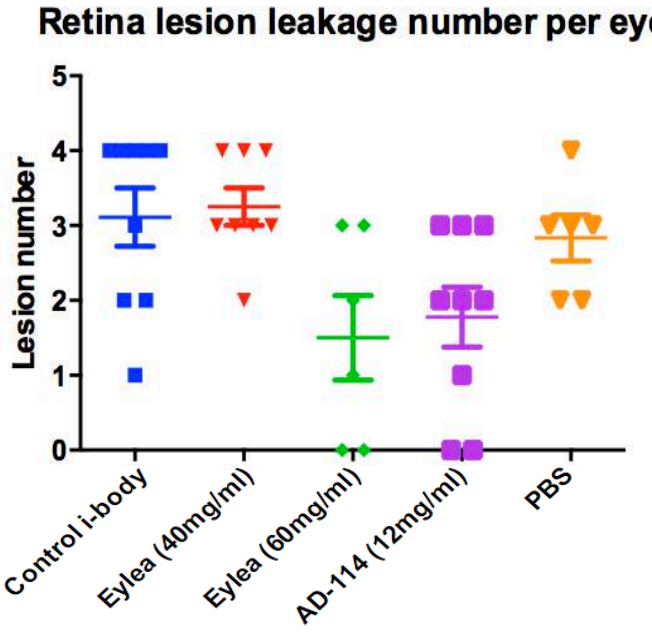
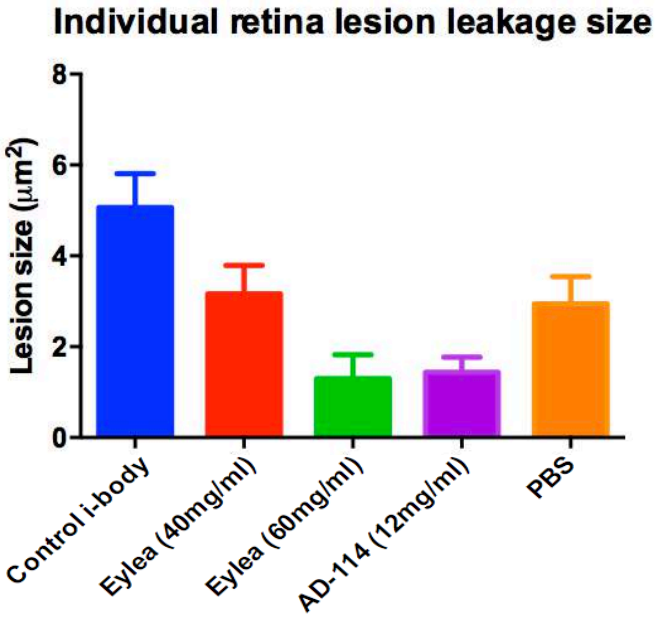
AD-114 reduces lesion size

- AD-114 is able to reduce the number and size of the lesion
- As expected Eylea was also able to reduce lesion number and size





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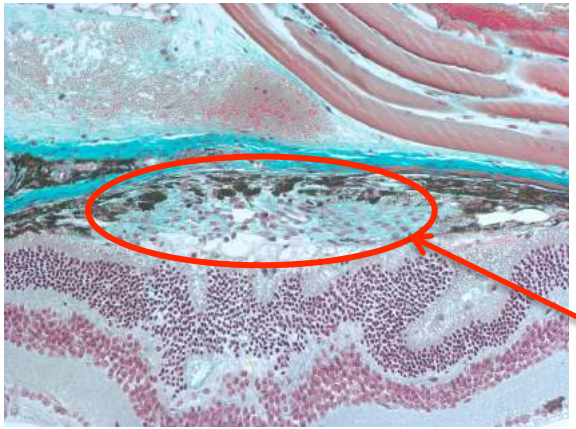
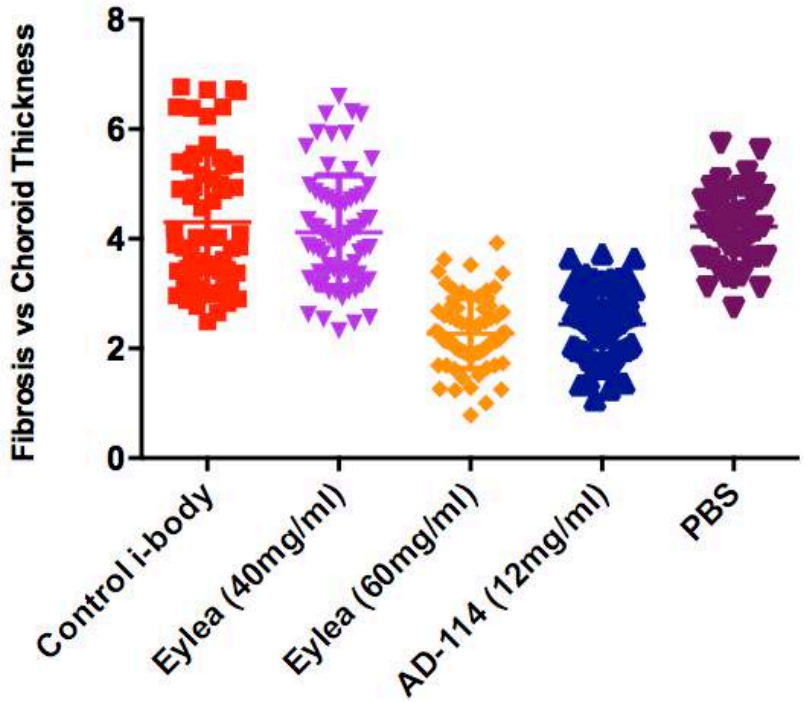
AD-114 reduces fibrosis

- AD-114 is able to significantly reduce fibrosis as measured by trichrome staining
- As expected Eylea was also able to reduce fibrosis in this assay



Fibrosis as measured by trichrome staining for collagen



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Model 2: laser induced subretinal haemorrhage

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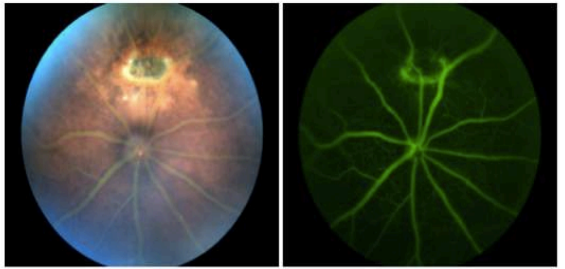
i-Body

Fluorescein angiography
Histology and mRNA

Laser: 532nm continuous wave laser



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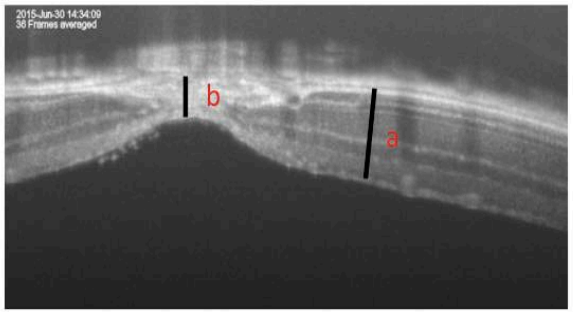

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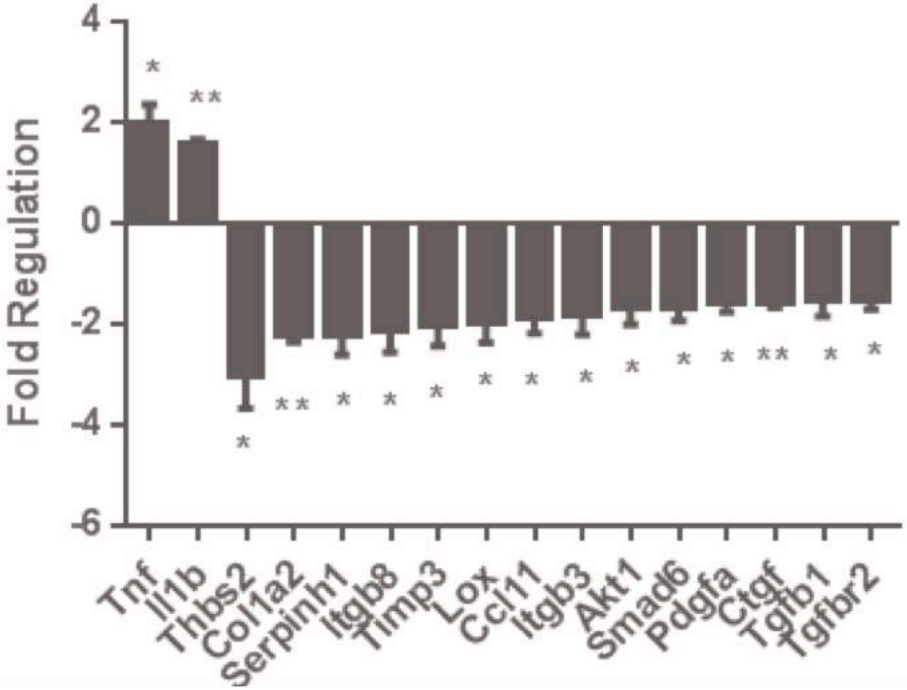
AD-114 prevents eye fibrosis in CNV model

- Mouse chorodial neo-vascularization model:
  - Induces subretinal haemorrhage
  - Contraction of retinal tissue
  - Alteration in microglia and glial response
  - Alteration in gene expression
- IVT injection of single dose of i-body
  - Reduces fibrosis gene expression

AD-114 reduces fibrotic gene expression in eye fibrosis mouse model



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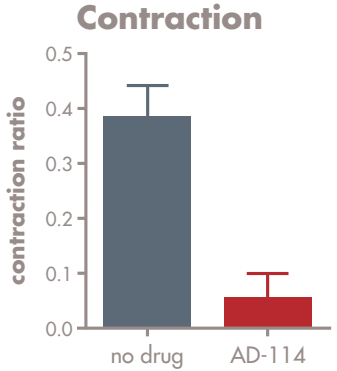
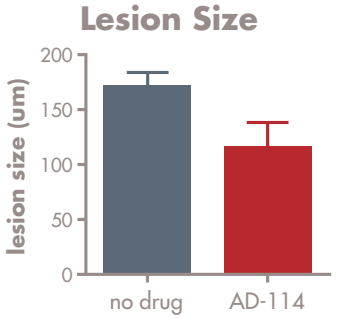
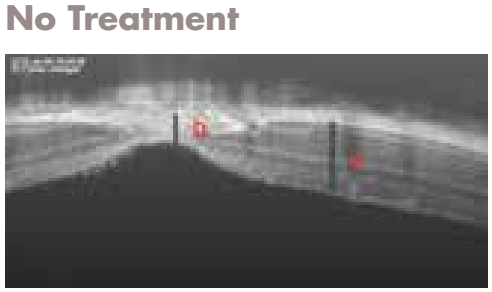
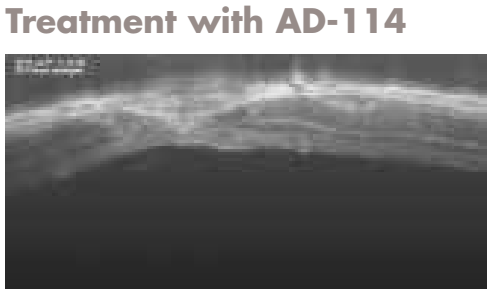
  - Reduces fibrosis gene expression
  - Improves retinal retraction and reduces lesion size

AD-114 reduces contraction and lesion size in eye fibrosis mouse model









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AD-114 a promising therapy for AMD

- AD-114 targets CXCR4: a novel mechanism of action for fibrosis making AD-114 a potential “first in class” therapy
- Anti-inflammatory activity in mouse air-pouch model
- Anti-fibrotic activity in Bleomycin mouse model of lung fibrosis
- Anti-fibrotic activity in mouse models of CNV and fibrosis
  - reduced the number and size of lesions
  - significantly reduced fibrosis and reduced pro-fibrotic gene expression
  - improves retinal retraction and reduces lesion size

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next generation protein therapeutics

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